

## Phylogeny of the *Graphium* butterflies inferred from nuclear 28S rDNA and mitochondrial ND5 gene sequences

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**Abstract** We examined the phylogenetic relationships of 40 species of the genus *Graphium* (Papilionidae: Leptocircini) with a few related genera belonging to the tribe Leptocircini. The analysis is based on comparisons of morphological classification and about 700 base pairs of nuclear 28S ribosomal DNA and 793 base pairs of the mitochondrial NADH dehydrogenase subunit 5 (ND5) gene. In both 28S rDNA, the ND5 and the 28S rDNA + ND5 phylogenetic trees of *Graphium* butterflies, the genus *Graphium* was monophyletic, and diversification occurred at almost the same time. Although the subgenera *Pazala*, *Pathysa* and *Arisbe* were monophyletic clusters, the subgenus *Graphium* was not. The 28S rDNA sequence lengths of the *eurypylus* group belonging to this subgenus (represented in this study by *G. doson*, *G. bathycles*, *G. chiron*, *G. evemon*, *G. leechi*, *G. eurypylus*) and *G. akikoe* (= *phidias*) to the subgenus *Pathysa* are 713 bases, and differed from those of the other *Graphium* butterflies which are 717 bases. The phylogenetic tree positions of the *eurypylus* group + *G. akikoe* did not cluster with other subgenera, but rather independently. Therefore, we assumed that the *eurypylus* group did not belong in the subgenus *Graphium*. The current classification leaves a certain ambiguity in the classification of the African *Graphium* species, either all the African *Graphium* species being classified into the subgenus *Arisbe* (Miller, 1987), or the African species being classified into two groups, the non-swordtailed species in the subgenus *Arisbe* and the swordtailed species in the subgenus *Graphium* (Hancock, 1983, 1993). The present study assumes that the African species should be assigned to two groups represented by Hancock, one being the non-swordtailed species in the subgenus *Arisbe* (represented in this study by *G. ridleyanus*, *G. latreillianus*, *G. angolatum*, *G. tynderaeus*, *G. leonidas*, *G. adamastor* and *G. schubotzi*) and the other being the swordtailed species in the subgenus *Graphium* (represented in this study by *G. policeses*).

**Key words** 28S ribosomal DNA, mitochondrial ND5 gene, phylogeny, *Graphium*, Leptocircini

### Introduction

The genus *Graphium* Scopoli, 1777 (type species: *Papilio sarpedon* Linnaeus, 1758), which belongs to the tribe Leptocircini (Papilionidae, Papilioninae), is widely distributed in the Oriental, Australian and Ethiopian regions, and has also spread into the southern area of the eastern Palaearctic region. This genus is not found in the New World, but the Leptocircini genus *Eurytides* Hübner, 1821 (type species: *Eurytides iphitas* Hübner, 1821), which contains the subgenus *Protesilaus* Swainson, 1832 (type species: *Papilio protesilaus* Linnaeus, 1758), is distributed in the Neotropical regions instead of the genus *Graphium* (recently, Möhn (2002) separated the new genus *Neographium* from the genus *Eurytides*). Other related genera

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belonging to this tribe, *Lamproptera* Gray, 1832 (type species: *Papilio curius* Fabricius, 1787), *Protographium* Munroe, 1961 (*Papilio leosthenes* Doubleday, 1846) and *Iphiclides* Hübner, 1819 (*Papilio podalirius* Linnaeus, 1758) are distributed in the Oriental, Australian and Palaearctic regions, respectively.

The relationships of the genus *Graphium* within the tribe Leptocircini and the interspecific relationships within *Graphium* have been previously discussed by many investigators including Ehrlich (1958), Munroe & Ehrlich (1960), Munroe (1961), Saigusa *et al.* (1977, 1982), Hancock (1983, 1993), Miller (1987) and Smith & Vane-Wright (2001). The current classification suggests that *Lamproptera* is most proximate to the genus *Graphium* (Hancock, 1983; Miller, 1987), while Ehrlich (1958) included *Lamproptera* in the genus *Graphium* based on the high tentorial crest. Munroe & Ehrlich (1960), on the other hand, state that the closest relative of *Graphium* is the genus *Iphiclides*.

The genus *Graphium* has been tentatively classified into four subgenera (Hancock, 1983): the eastern Palaearctic subgenus *Pazala* Moore, 1888 (type species: *Papilio glycerion* Gray, 1831), the Indo-Australian subgenus *Pathysa* Reakirt, 1865 (type species: *Papilio antiphates* Cramer, 1775), the African subgenus *Arisbe* Hübner, 1819 (type species: *Papilio leonidas* Fabricius, 1793) and the Asian subgenus *Graphium* Scopoli (Table 1).

In the current classification of the subgenus *Graphium* based on Saigusa *et al.* (1977, 1982), the subgenus *Graphium* consists of the *sarpedon* group, the *agamemnon* group and the *eurypylus* group. However, Saigusa *et al.* (1977, 1982) did not specifically state that the subgenus *Graphium* is monophyletic. Miller (1987) also suggested that he could not find autapomorphic characteristics to support the subgenus *Graphium*.

Munroe (1961) classified all African species into the subgenus *Arisbe*, based on the rim of the valva of the male genitalia having stout setae concentrated on the disto-ventral half of the dorsal process, but not modified into spines in either region. Miller (1987) also classified all African species into *Arisbe*, based on the lateral lobes of the female ostium, which are covered with a mat of short setae and have a fuzzy appearance throughout the subgenus *Arisbe*. However, Hancock (1983, 1993) classified the African species into the Afrotropical swordtailed species in *Graphium*, and the non-swordtailed species in *Arisbe*, despite the assertions of Munroe (1961) and Miller (1987) that all African *Graphium* butterflies should be classified into the subgenus *Arisbe*. The recent Smith & Vane-Wright (2001) classification of the Afrotropical *Graphium* butterflies with a few Indo-Australian butterflies based on the morphological characters did not involve four subgenera, and it showed that various species formed small groups and had complicated phylogenetic relationships.

Munroe (1961) further suggested that the *eurous* group, placed by later authors in the subgenus *Pazala*, belongs to *Pathysa*, based on the complexity of the male genitalia. He does not recommend separating this group as the fourth subgenus because of significant affinities between *Pazala* and *Pathysa*. Hancock (1983) pointed out that the most primitive subgenus, *Pazala*, retains the primitive banded pattern, complex male clasper and blue hindwing scales, while in the slightly more advanced *Pathysa*, the pattern is either primitive or mimetic, the clasper less complex and blue scales absent. Miller (1987) also agreed that the subgenus *Pazala* is plesiomorphic to the rest of the genus *Graphium*.

In spite of this seeming agreement, Igarashi's study of immature stages (1984) indicates that the genus *Lamproptera* is more closely related to both the subgenera *Graphium* and *Arisbe* than to either *Pazala* or *Pathysa*. Igarashi treated *Pazala* and *Pathysa* as two genera, and other researchers (*e. g.* D'Abrera, 1982) have also recognized *Pazala* as a separate genus.

Moreover, a cladistic study by Tsukada & Nishiyama (1980) suggested that *Graphium akikoe* Morita & Shinkai\* (= *G. phidias* (Oberthür)) would belong in a new subgenus, not belonging to *Pathysa* shown by Hancock (1983) or Collins & Morris (1985). Niculescu (1977) proposed a new genus, *Klingizia* (a replacement name, *Klingiziana* Niculescu, 1989) for *Graphium weiskei* (Ribbe), but this proposal has not been accepted by the subsequent authors (Parsons, 1999). As mentioned above, subgeneric classifications or grouping of species within the genus *Graphium* are not yet clear.

In the present study, we examined the nucleotide sequences of a part of the nuclear 28S ribosomal DNA (about 700 base pairs) and a part of the mitochondrial gene encoding ND5 (793 base pairs) of *Graphium* butterflies. The ND5 gene shows a fairly rapid evolution and is one of the most useful mitochondrial genes for investigating the phylogenetic relationships among related groups (Su *et al.*, 1996a, b; Yagi *et al.*, 1999; Kim *et al.*, 2000; Makita *et al.*, 2000). Although the 28S rDNA shows a slower evolution than the ND5 gene, the 28S rDNA phylogeny is mostly consistent with the ND5 gene phylogeny of the *Leptocarabus* ground beetles (Kim *et al.*, 2000). Therefore, we used 28S rDNA for the tribe Leptocircini groups and ND5 for the genus *Graphium*. The molecular phylogeny indicated the evolution and phylogeny of the genus *Graphium*.

## Materials and methods

### Samples and DNA extraction

The names and localities of the specimens used in this study are shown in Table 1, including their accession numbers assigned by the DDBJ/EMBL/GenBank; representative species are shown in Fig. 1. A total of 40 *Graphium* butterfly species were analyzed, including several species of each subgenus of the genus *Graphium*, as well as species from three related genera belonging to the tribe Leptocircini: the genus *Eurytides* (*E. helios* Rothschild & Jordan), *Iphiclides* (*I. podalirius* (Linnaeus)), and *Lamproptera* (*L. meges* (Zinken-Sommer) and *L. curius* (Fabricius)). One species of the genus *Teinopalpus* Hope (*T. imperialis* Hope) was used as an outgroup.

All butterflies were collected by the authors or supplied by entomologists (see acknowledgments). The butterflies collected in Japan, Korea and Myanmar were immersed in 95% ethanol and stored at  $-20^{\circ}\text{C}$  before use, and those collected in other areas, China, Malaysia, Indonesia and so on, or rare or precious samples were kept as mounted dry specimens. DNA was extracted from muscles of the thorax or legs, using a QIAamp DNeasy Kit (Qiagen, Inc., Valencia, CA, USA). (Note; many of the rare or precious samples used in this study were purchased from a dealer who obtained permission, and were collected before 1996).

### PCR amplification

Extracted total DNA was used as a template for 28S rDNA and ND5 DNA amplification

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\* *Papilio phidias* Oberthür, 1906 is a primary homonym of *Papilio phidias* Linnaeus, 1758, and cannot be used as the scientific name for the well-known *Graphium* species in question. We use *Graphium akikoe* Morita & Shinkai, 1996 as its valid name. Although *akikoe* was described as a subspecies of *G. phidias* from Lak Sao, Laos, Masui and Uehara (1996) suggested that there is no big difference between the specimens from Laos and Vietnam, from which *phidias* Oberthür, 1906 was originally described.

Table 1. List of species of the genus *Graphium* and other related genera used in this study.

genus subgenus species group species	28S	ND5	locality	DDBJ/EMBL/GenBank accession no. (28S/ND5)
<i>Graphium</i>				
<i>Pathysa</i>				
<i>antiphates</i> group				
<i>agetes</i> (Westwood)	+	+	Laos	AB059684/AB059486
	+	+	Malaysia	AB059685/AB059485
<i>aristeus</i> (Cramer)	—	+	Malaysia	—/AB059487
<i>stratotes</i> (Grose-Smith)	—	+	Sabah, Borneo	—/AB059491
<i>androcles</i> (Boisduval)	+	+	Sulawesi, Indonesia	AB088638/AB088644
<i>dorcus</i> (de Haan)	—	+	Sulawesi, Indonesia	—/AB088645
<i>antiphates</i> (Cramer)	+	+	Laos	AB059690/AB059494
	—	+	Cameron Highland, Malaysia	—/AB059495
	+	+	Borneo	AB059691/AB059496
<i>macareus</i> group				
<i>delesserti</i> (Guérin-Méneville)	—	+	Borneo	—/AB059488
<i>macareus</i> (Godart)	+	+	Laos	AB059686/AB059489
	+	+	Borneo	AB059687/AB059490
<i>thule</i> (Wallace)	+	+	Timuka, W. Irian, Indonesia	AB059688/AB059492
<i>xenocles</i> (Doubleday)	+	+	Tam Dao, Hanoi, Vietnam	AB059689/AB059493
<i>Pazala</i>				
<i>mandarinum</i> (Oberthür) <sup>1)</sup>	+	+	Mt Victoria, Chin, Myanmar 1	AB059725/AB059533
	+	+	Mt Victoria, Chin, Myanmar 2	AB059726/AB059534
<i>incertum</i> (Bang-Haas)	+	+	Yunnan, China	AB059727/AB059535
<i>hoenei</i> (Mell) <sup>2)</sup>	+	+	Sichuan, China	AB059728/AB059536
<i>tamerlanum</i> (Oberthür)	+	+	Gansu, China	AB059729/AB059537
<i>eurous</i> (Leech)	+	+	Taiwan	AB059730/AB059538
<i>Graphium</i>				
<i>akikoe</i> Morita & Shinkai <sup>3)</sup>	+	+	Laos	AB059701/AB059505
<i>eurypylus</i> group				
<i>evemon</i> (Boisduval)	+	+	Cameron Highland, Malaysia 1	AB059692/AB059497
		+	+ Cameron Highland, Malaysia 2	AB059693/AB059497
<i>doson</i> (C. & R. Felder)	+	+	Laos 1	AB059694/AB059498
	+	+	Laos 2	AB059695/AB059499
	+	+	Taiwan 1	AB059696/AB059500
	+	+	Taiwan 2	AB059697/AB059501
<i>chiron</i> (Wallace)	+	+	Zi ya dum, Myanmar	AB059698/AB059502
<i>bathycles</i> (Zinken-Sommer)	+	+	Cameron Highland, Malaysia	AB059699/AB059503
<i>leechi</i> (Rothschild)	+	+	Jingning, Zhejiang, China	AB088639/AB088646
<i>eurypylus</i> (Linnaeus)	+	—	Putao, Kachin, Myanmar	AB059700/—
<i>agamemnon</i> group				
<i>agamemnon</i> (Linnaeus)	+	+	Bali Is., Indonesia	AB059702/AB059506
	+	+	Cameron Highland Malaysia 1	AB059703/AB059507
	+	+	Cameron Highland Malaysia 2	AB059704/AB059508
	+	+	Laos	AB059705/AB059509
	+	+	Taiwan	AB059706/AB059510
	+	+	Borneo	AB059707/AB059511
<i>wallacei</i> group				
<i>wallacei</i> (Hewitson)	+	+	New Britain Is., PNG	AB059708/AB059512
<i>sarpedon</i> group				
<i>batjanense</i> Okano	+	+	Bachan Is., Indonesia	AB059709/AB059513
<i>weiskei</i> (Ribbe)	+	+	PNG	AB059710/AB059514
<i>macleayanum</i> (Leach)	+	—	Dungog, Australia	AB088643/—
<i>codrus</i> (Cramer)	+	+	Malaita Is, Solomons	AB059711/AB059515
<i>empedovanum</i> (Corbet)	+	+	Java, Indonesia 1	AB059712/AB059516
	—	+	Java, Indonesia 2	—/AB059517
<i>sarpedon</i> (Linnaeus)	+	+	Bali Is., Indonesia	AB059713/AB059518
	+	+	Taiwan 1	AB059714/AB059519

	+	+	Taiwan 2	AB059715/AB059520
	+	+	Chiba, Japan	AB059716/AB059521
	+	+	Yokosuka, Japan	AB060636/AB059522
	+	+	Cameron Highland, Malaysia 1	AB059717/AB059523
	+	+	Cameron Highland, Malaysia 2	AB059718/AB059524
	+	+	Cameron Highland, Malaysia 3	AB059719/AB059525
	+	+	Laos	AB059720/AB059526
<i>milon</i> C. & R. Felder	+	+	Sulawesi, Indonesia	AB059721/AB059527
	—	+	Buru Is., Indonesia	—/AB059528
<i>cloanthus</i> Westwood	+	+	Taiwan	AB059722/AB059529
	+	+	Laos	AB059723/AB059530
	—	+	Sichuan, China	—/AB059531
<i>policenes</i> group				
<i>policenes</i> (Cramer)	+	+	Congo	AB059724/AB059532
<i>Arisbe</i>				
<i>ridleyanus</i> group				
<i>ridleyanus</i> (White)	+	+	Nola, RCA	AB059731/AB059539
<i>tynderaeus</i> group				
<i>latreillianus</i> (Godart)	+	+	Camerun	AB059732/AB059540
<i>tynderaeus</i> (Fabricius)	+	+	Bungui, RCA	AB088642/AB088648
<i>angolanum</i> group				
<i>angolanum</i> (Goeze) <sup>4)</sup>	+	+	Bungui, RCA	AB088640/AB088647
<i>leonidas</i> group				
<i>leonidas</i> (Fabricius)	—	+	Bungui, RCA	—/AB059541
<i>adamastor</i> group				
<i>adamastor</i> (Honrath)	+	+	Bungui, RCA	AB088641/AB088649
<i>schubotzi</i> (Schultze) <sup>5)</sup>	—	+	Bungui, RCA	—/AB088650
<i>Lamproptera</i>				
<i>meges</i> (Zinken-Sommer)	+	+	Cameron Highland, Malaysia 1	AB059733/AB059542
	+	+	Cameron Highland, Malaysia 2	AB059734/AB059543
	+	+	Cameron Highland, Malaysia 3	AB059735/AB059544
<i>curius</i> (Fabricius)	+	+	Ziya dum, Myanmar	AB059736/AB059545
<i>Iphiclides</i>				
<i>podalirius</i> (Linnaeus)	+	+	Czech Republic	AB059737/AB059546
<i>Eurytides</i>				
<i>Protesilaus</i>				
<i>protesilaus</i> group				
<i>helios</i> (Rothschild & Jordan)	+	+	Minas Gerais, Brazil	AB059738/AB059547
<i>Teinopalpus</i>				
<i>imperialis</i> Hope	+	+	N. Vietnam	AB059739/AB059548

Arrangement of species follows Hancock (1983) except for *G. akikoe* (= *phidias*).

- 1) *G. glycerion* (Gray, 1831) is a junior primary homonym of *Papilio glycerion* Borkhausen, 1788.
- 2) *G. sichuanica* (Koiwaya, 1993) is a junior subjective synonym.
- 3) *G. phidias* (Oberthür, 1906) is a junior primary homonym of *Papilio phidias* Linnaeus, 1758.
- 4) *G. pylades* (Fabricius, 1793) is a junior primary homonym of *Papilio pylades* Stoll, 1782.
- 5) *G. odin* (Strand, 1910) is a junior primary homonym of *Papilio odin* Fabricius, 1793.

by polymerase chain reaction (PCR), following the method described by Saiki *et al.* (1988). The PCR primers for the 28S rDNA gene were employed following the method described by Kim *et al.* (2000). Other PCR primers for 28S rDNA were based on *Graphium* sequences; these were the following: 28rSF (5'-GAC TAC CCC TGA WTT WAA GCA T-3'), 28rSR (5'-GAC TCC TTG GTC CGT GTT TC-3'), and the sequence primers 28sF1 (5'-AGT AGC GGC GAG CGA ACA GGA A-3'), 28sF2 (5'-GCG AAA CTC GAA TGA ACG AAC GG-3'), 28sR1 (5'-CCC GAA ACT GAA TCA TCG CCG AC-3'), and 28sR2 (5'-CSG ACR TCG AAC GGG TCG CGA TG-3'). The PCR primers for the ND5 gene

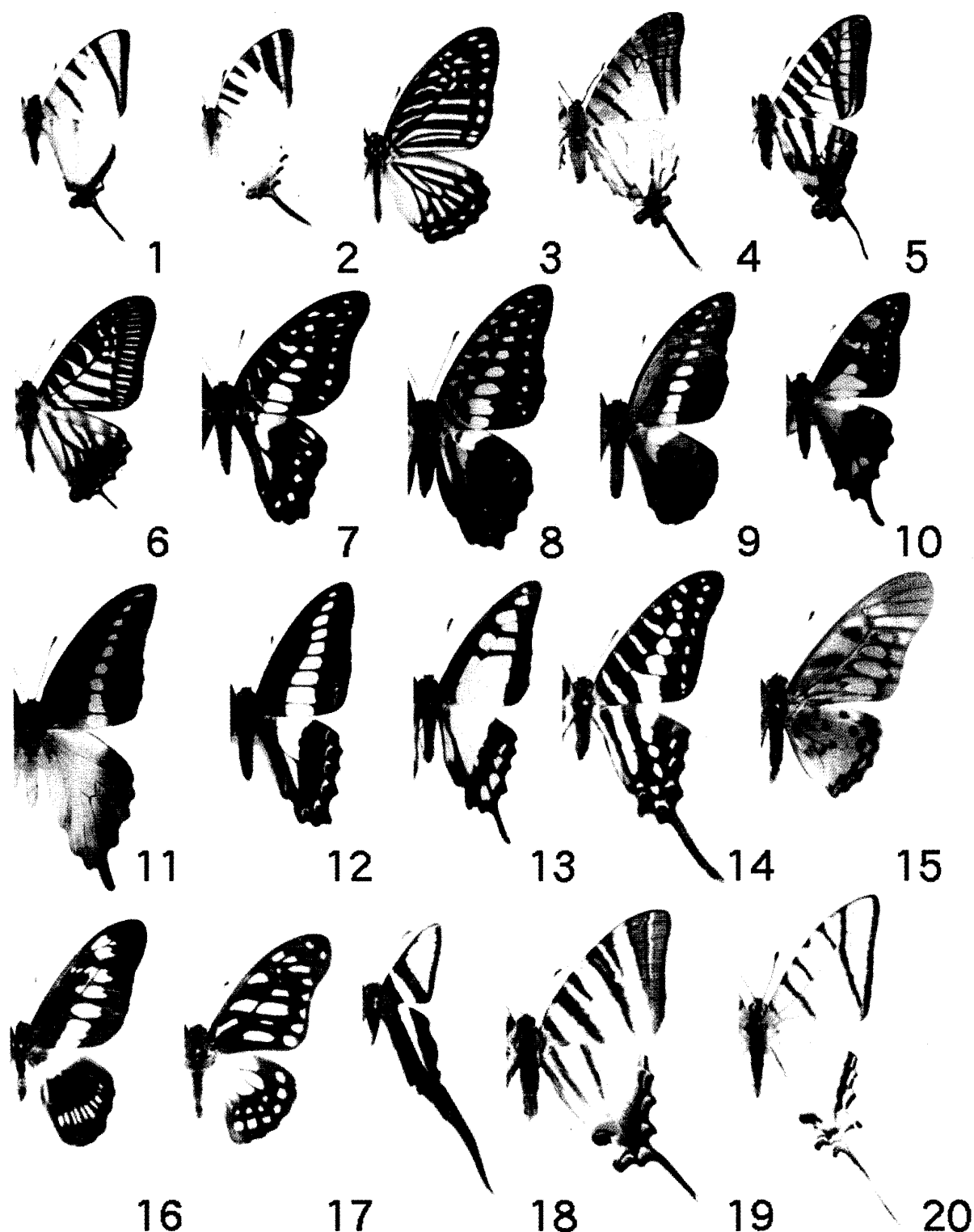


Fig. 1. Representative species of the genus *Graphium* and the tribe Leptocircini used in this study. 1. *Graphium agetes*. 2. *G. antiphates*. 3. *G. macareus*. 4. *G. mandarinum*. 5. *G. eurous*. 6. *G. akikoe* (= *phidias*). 7. *G. doson*. 8. *G. agamemnon*. 9. *G. wallacei*. 10. *G. weiskei*. 11. *G. codrus*. 12. *G. sarpedon*. 13. *G. cloanthus*. 14. *G. polices*. 15. *G. ridleyanus*. 16. *G. latreillianus*. 17. *G. leonidas*. 18. *Lamproptera meges*. 19. *Iphiclides podalirius*. 20. *Eurytides helios*.

were employed following the method described by Su *et al.* (1996a, b) and Yagi *et al.* (1999). Other PCR primers for the ND5 gene were based on *Graphium* sequences: C2GR (5'-KCN GGR TTT TAT TCW AAR GAT-3'), A4G (5'-GTR TAA WAT ATA GTY ARH CCW GTW G-3'), and DR2G (5'-GTW GAT AAA TTA GGT ATA AAT CAY A-3'). PCR amplification was carried out in 50  $\mu$ l of a solution containing 1  $\mu$ g of template DNA, 1 pmol/ $\mu$ l of each primer, and 1.25 U of *TaKaRa Ex Taq* (Takara, Shiga, Japan). The amplification comprised 35 cycles of denaturation at 94°C for 30 sec, primer annealing at 52°C for 30 sec and primer extension at 72°C for 1 min, or was conducted according to the method described by Taylor *et al.* (1993). Taylor's PCR method was used primarily for dry specimens.

### Direct sequencing of PCR-amplified single stranded DNAs

The PCR products were purified using a QIAquick Gel Extraction Kit or QIAquick PCR Purification Kit (Qiagen). Direct sequencing was performed by the dideoxy chain termination method (Sanger *et al.*, 1977) using a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems Japan, Ltd, Tokyo, Japan). The nucleotide sequences were determined using an ABI PRISM 377 Sequencer (Applied Biosystems).

### Phylogenetic analysis

The 28S rDNA and ND5 DNA sequences were aligned using the Clustal X program (Thompson *et al.*, 1997), and were then manually adjusted by eye to minimize gaps. Phylogenetic analysis was performed by two methods: the neighbor-joining (NJ) method (Saitou & Nei, 1987) and the maximum parsimony (MP) method. Both methods with a bootstrap test (Felsenstein, 1985) were carried out with the PAUP\* 4.0b10 program (Swofford, 2000) and the heuristic parsimony analysis used both with all characters having equal weight, stepwise sequence addition, addition sequence of 1,000 random replicates, tree-bisection-reconnection (TBR) branch-swapping, and the MULTREES option. The bootstrap values are based on 1,000 replicates. Gaps in the alignments were excluded from the phylogenetic analysis. The evolutionary distances (D) were computed by Kimura's two-parameter method (Kimura, 1980). The gene sequences of the genus *Teinopalpus* (*T. imperialis*) were used as an outgroup.

## Results

### Nucleotide sequences

Partial sequences of the nuclear 28S rDNA (about 700 base pairs) and the mitochondrial ND5 (793 base pairs) were determined, although not all taxa were sequenced successfully (e. g., *Graphium eurypylus* (Linnaeus) for ND5). A few deletions and length variations were found in each genus as well as in the *Graphium* 28S rDNA sequences. Certain length variations of the 28S rDNA were found within the genus *Graphium*, specifically, *Graphium doson* (C. and R. Felder), *G. bathycles* (Zinken-Sommer), *G. chiron* (Wallace), *G. evemon* (Boisduval), *G. leechi* (Rothschild), and *G. eurypylus*, which were classified into the *eurypylus* group in the subgenus *Graphium* and *G. akikoeae*, showed 713 bases, but other *Graphium* species including *Pathysa*, *Pazala* and *Arisbe* showed 717. The genera of the tribe Leptocircini showed the following numbers of bases: *Eurytides*, 724; *Iphiclides*, 718; *Lamproptera*, 718; and *Teinopalpus*, 728. There were no insertions/deletions and no length variations for

Table 2. Pairwise Sequence Divergence (by Kimura's Two-Parameters Methods) for 28S rDNA examined in this study

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	-	n.e.	n.e.	0.00	n.e.	0.14	0.00	0.14	n.e.	0.42	1.56	1.85	1.56	1.13	1.56	4.18	4.18	4.18	4.03	4.03	4.18	4.33
2	8.69	-	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
3	10.17	9.86	-	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
4	9.87	9.12	5.61	-	n.e.	0.14	0.00	0.14	n.e.	0.42	1.56	1.85	1.56	1.13	1.56	4.18	4.18	4.18	4.03	4.03	4.18	4.33
5	6.59	8.69	9.43	10.00	-	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
6	8.69	8.11	9.28	8.11	9.27	-	0.14	0.28	n.e.	0.56	1.70	1.99	1.70	1.27	1.70	4.33	4.33	4.33	4.18	4.18	4.33	4.48
7	10.00	9.99	4.09	5.05	10.00	8.68	-	0.14	n.e.	0.42	1.56	1.85	1.56	1.13	1.56	4.18	4.18	4.18	4.03	4.03	4.18	4.33
8	9.71	5.46	10.31	9.27	10.30	8.11	10.44	-	n.e.	0.56	1.70	1.70	1.70	1.27	1.42	4.33	4.18	4.33	4.18	4.18	4.33	4.48
9	11.61	9.26	12.22	12.06	10.72	9.70	12.21	10.42	-	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
10	11.04	8.82	11.18	9.26	11.47	9.40	10.88	10.00	8.12	-	1.85	2.14	1.85	1.56	1.85	4.03	4.03	4.03	3.89	3.89	4.03	4.18
11	12.70	13.13	13.78	12.40	13.47	12.22	13.14	13.00	13.75	12.56	-	0.28	0.00	0.42	0.28	3.44	3.74	3.74	3.59	3.59	3.74	3.89
12	10.14	10.57	10.73	10.00	11.03	10.57	10.43	11.48	11.76	10.29	5.77	-	0.28	0.71	0.28	3.74	3.89	4.03	3.89	3.89	4.03	4.18
13	11.93	12.22	13.15	11.93	12.38	11.01	12.98	11.17	12.67	11.18	3.30	5.21	-	0.42	0.28	3.44	3.74	3.74	3.59	3.59	3.74	3.89
14	11.19	11.17	11.64	9.55	11.04	9.55	11.47	10.88	11.47	11.19	5.90	4.37	5.90	-	0.42	3.89	4.18	4.18	4.03	4.03	4.18	4.33
15	10.14	10.13	10.74	8.68	10.00	8.68	10.58	10.14	10.87	10.44	5.77	3.96	5.49	1.96	-	3.74	3.89	4.03	3.89	3.89	4.03	4.18
16	11.62	10.72	11.19	10.74	11.33	10.57	10.73	11.04	11.61	11.18	13.15	10.44	11.77	11.04	9.84	-	0.99	0.99	0.85	0.85	0.99	1.13
17	10.88	9.70	10.15	9.27	10.44	9.26	10.58	10.00	10.88	9.85	10.30	8.69	8.97	9.28	8.55	0.00	-	0.56	0.42	0.42	0.56	0.71
18	11.80	10.15	10.94	9.59	11.05	10.90	10.75	11.19	10.91	10.47	9.14	9.87	9.00	8.27	7.99	6.48	-	0.14	0.14	0.00	0.14	0.28
19	11.47	9.85	10.60	9.56	11.05	9.70	10.43	9.85	10.73	10.45	10.16	8.69	8.69	8.70	7.69	8.84	4.92	4.94	-	0.00	0.14	0.28
20	11.78	9.85	11.20	9.71	11.20	9.84	11.18	9.55	10.73	10.30	10.92	9.13	8.83	8.85	8.12	8.84	5.06	4.52	1.96	-	0.14	0.28
21	11.04	9.85	9.71	9.42	10.90	9.41	10.00	10.29	10.73	10.60	10.46	8.55	8.98	8.70	7.69	8.84	5.20	5.65	3.83	4.25	-	0.14
22	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	-
23	10.57	9.11	10.72	10.57	10.13	9.40	11.01	9.84	9.11	9.69	11.05	8.39	10.15	8.70	8.12	8.70	8.83	9.72	8.40	8.69	8.11	n.e.
24	12.22	10.42	11.77	10.42	11.76	9.84	11.16	10.57	11.16	10.72	10.60	8.98	9.27	9.12	8.11	10.28	9.56	9.85	9.41	9.41	8.68	n.e.
25	13.21	11.57	13.52	12.01	12.17	11.13	13.51	11.32	12.46	11.13	11.59	9.97	10.55	10.13	9.69	11.00	8.41	10.15	9.40	9.69	10.13	n.e.
26	12.97	11.46	13.13	12.21	12.22	10.42	12.97	11.62	11.76	11.77	11.04	9.84	9.55	9.85	9.12	11.33	8.40	9.72	8.83	8.83	8.98	n.e.
27	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
28	13.27	11.46	12.69	11.46	12.21	11.02	13.43	10.87	11.16	12.51	12.25	11.33	11.03	11.03	10.28	12.37	10.43	11.03	10.58	10.28	10.28	n.e.
29	14.20	14.51	14.24	13.92	14.69	13.74	15.01	14.35	13.43	14.37	13.03	11.80	11.64	12.41	12.25	13.31	12.54	12.55	12.69	12.54	12.23	n.e.
30	12.36	11.01	12.82	11.17	11.76	11.31	12.97	10.87	12.82	13.90	13.16	10.58	11.47	10.74	9.85	12.37	10.88	11.64	10.44	10.74	9.99	n.e.
31	12.51	11.46	12.98	10.72	12.07	11.31	13.13	11.02	12.36	13.29	13.32	11.18	11.78	10.89	10.59	13.14	11.03	11.64	11.34	11.34	10.73	n.e.
32	13.74	12.36	13.13	11.16	12.51	11.17	12.66	11.62	11.31	11.47	13.00	10.88	11.32	9.99	9.40	11.16	10.28	11.04	9.85	10.59	9.70	n.e.
33	14.20	12.51	13.28	11.61	12.82	11.17	12.82	11.77	11.46	11.62	13.45	11.32	11.62	10.73	10.13	11.61	10.72	11.49	10.28	10.88	10.44	n.e.
34	13.12	11.31	12.67	12.07	12.36	12.21	12.66	11.01	12.22	11.91	13.92	11.47	11.92	12.23	11.32	11.91	10.29	12.26	10.73	11.03	11.33	n.e.
35	11.02	9.84	11.19	10.58	10.73	9.26	11.02	9.85	10.42	11.02	12.08	9.98	10.87	9.26	9.26	10.14	9.41	10.62	8.69	9.13	9.28	n.e.
36	12.36	11.16	13.12	12.36	12.06	11.02	12.97	11.61	12.36	12.97	13.14	10.57	11.16	11.32	10.87	12.66	9.69	11.93	10.72	10.72	11.02	n.e.
37	11.76	9.69	10.30	9.26	10.58	9.55	10.43	9.85	10.43	10.58	10.59	8.82	9.70	9.12	8.83	9.70	7.68	9.59	8.26	8.70	8.69	n.e.
38	10.73	8.39	10.45	9.85	10.59	8.68	10.72	9.85	9.55	10.14	11.50	8.54	10.45	9.42	8.83	9.85	8.26	9.29	8.40	8.84	8.55	n.e.
39	10.58	8.97	10.15	9.84	9.11	7.96	10.13	9.40	10.43	9.71	11.18	9.41	10.44	8.69	8.40	9.26	7.97	9.59	8.69	8.99	8.70	n.e.
40	10.87	9.26	10.59	9.70	10.14	8.39	9.84	9.69	9.84	9.26	11.18	8.83	10.74	8.84	8.25	9.12	8.69	9.14	8.40	8.69	8.70	n.e.
41	13.43	11.76	11.91	11.31	11.76	11.92	11.16	12.06	11.61	11.46	12.83	10.28	11.02	10.87	9.98	9.40	8.39	10.30	8.68	9.12	8.68	n.e.
42	14.35	12.67	12.66	12.21	12.21	13.43	12.06	13.27	12.82	11.91	13.60	11.02	12.22	11.47	10.87	10.58	9.26	11.18	9.40	9.70	10.14	n.e.
43	14.97	13.74	14.20	12.97	13.43	13.28	14.51	13.73	13.27	12.21	13.59	12.51	12.82	12.52	11.31	13.58	11.76	12.84	12.07	12.22	12.07	n.e.
44	12.06	11.03	11.31	10.72	11.91	12.07	11.32	11.61	12.67	12.51	12.70	10.57	11.32	11.17	10.28	12.66	9.98	11.02	10.28	10.28	10.72	n.e.
45	16.09	14.67	14.22	13.90	13.90	15.30	15.14	15.15	15.29	13.74	15.93	14.99	14.82	15.15	14.21	13.77	13.34	14.28	12.71	13.02	13.01	n.e.

Note. - The numbers above the diagonal are for 28S rDNA, and those below the diagonal are for 1. *G. agetes* Laos, 2. *G. aristus* Malaysia, 3. *G. delesserti* Borneo, 4. *G. macareus* Laos, 5. *G. dorcus* Sulawesi, 10. *G. antiphates* Laos, 11. *G. mandarinum* Myanmar1, 12. *G. incertum* Yun-Laos, 17. *G. evemon* Cameron, 18. *G. doson* Laos1, 19. *G. chiron* Myanmar, 20. *G. bathycles lacei* PNG, 25. *G. batjanensis* Bachan, 26. *G. weiskei* PNG, 27. *G. macleayanum* Australia, 28. *G. cloanthus* Taiwan, 33. *G. policenes* Congo, 34. *G. ridleyanus* Nola RCA, 35. *G. latreillianus* gui RCA, 39. *G. adamastor* Bungui RCA, 40. *G. schubotzi* Bungui RCA, 41. *L. meges* Camer-

the ND5 gene in any of the species examined. The G+C content was 59.1–60.4% for 28S rDNA and 16.5–18.6% for the ND5 gene in the analyzed *Graphium* species. The maximum sequence divergences of the 28S rDNA and the ND5 genes were 5.23% and 15.46% for the *Graphium* species (Table 2). The 28S rDNA was found to have evolved about one third as fast as the ND5 gene.

### Phylogenetic tree of the *Graphium* butterflies

Figs 2, 3 and 4 show the NJ and MP phylogenetic trees of 28S rDNA, the ND5 gene and 28S+ND5, respectively. One *Teinopalpus* species was used as an outgroup. Gaps in the alignments were excluded from phylogenetic analysis, and both gap-excluded and unexcluded trees showed almost identical phylogenetic relationships (gap-unexcluded trees are not shown). Each phylogenetic tree indicates that the genus *Graphium* including *Pathysa*, *Pazala* and *Arisbe* is monophyletic, however, the relationships between *Graphium* and other



and ND5 of representative *Graphium* species and related genera of the tribe Graphiini that were

23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
1.28	1.13	1.13	1.13	1.13	2.14	2.87	1.13	1.13	1.42	1.56	0.71	0.71	0.71	0.99	n.e.	0.85	n.e.	4.04	4.04	3.59	3.30	5.23
n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
1.28	1.13	1.13	1.13	1.13	2.14	2.87	1.13	1.13	1.42	1.56	0.71	0.71	0.71	0.99	n.e.	0.85	n.e.	4.04	4.04	3.59	3.30	5.23
n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
1.42	1.28	1.27	1.27	1.27	2.28	3.02	1.28	1.28	1.56	1.71	0.85	0.85	0.85	1.13	n.e.	0.99	n.e.	4.04	4.04	3.74	3.45	5.23
1.28	1.13	1.13	1.13	1.13	2.14	2.87	1.13	1.13	1.42	1.56	0.71	0.71	0.71	0.99	n.e.	0.85	n.e.	4.04	4.04	3.59	3.30	5.23
1.42	1.28	1.27	1.27	1.27	2.14	3.02	1.28	1.28	1.56	1.71	0.85	0.85	0.85	1.13	n.e.	0.99	n.e.	4.18	4.18	3.74	3.45	5.38
n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
1.13	0.71	0.98	0.99	0.99	1.99	2.87	0.85	0.85	1.13	1.27	0.99	0.85	0.99	1.27	n.e.	1.13	n.e.	3.89	3.89	3.74	3.45	5.53
1.42	1.70	1.41	1.56	1.56	2.86	3.30	1.99	1.99	2.14	2.28	1.13	1.56	1.13	1.42	n.e.	1.27	n.e.	4.63	4.63	3.30	3.89	5.23
1.70	1.99	1.69	1.85	1.85	3.01	3.60	2.28	2.28	2.43	2.57	1.42	1.85	1.42	1.70	n.e.	1.56	n.e.	4.93	4.93	3.59	4.18	5.53
1.42	1.70	1.41	1.56	1.56	2.86	3.30	1.99	1.99	2.14	2.28	1.13	1.56	1.13	1.42	n.e.	1.27	n.e.	4.63	4.63	3.30	3.89	5.23
1.56	1.70	1.27	1.42	1.42	2.71	3.30	1.70	1.70	1.85	1.99	1.27	1.56	1.27	1.56	n.e.	1.42	n.e.	4.33	4.33	3.59	3.59	5.08
1.70	1.99	1.41	1.56	1.56	2.71	3.60	1.99	1.99	2.14	2.28	1.42	1.85	1.42	1.70	n.e.	1.56	n.e.	4.63	4.63	3.59	3.89	5.23
3.15	3.59	3.23	3.30	3.30	4.48	5.08	3.44	3.44	3.44	3.59	3.74	4.18	3.74	4.03	n.e.	3.89	n.e.	6.29	6.29	5.38	6.14	6.90
3.15	3.59	3.44	3.45	3.45	4.03	4.93	3.44	3.44	3.45	3.59	3.74	4.48	3.74	4.18	n.e.	4.03	n.e.	5.98	5.98	5.23	5.68	6.90
3.15	3.59	3.59	3.59	3.59	4.48	5.08	3.44	3.44	3.44	3.59	4.03	4.48	4.03	4.33	n.e.	4.18	n.e.	6.29	6.29	5.23	5.99	6.90
3.01	3.44	3.45	3.45	3.45	4.33	4.93	3.30	3.30	3.30	3.44	3.89	4.33	3.89	4.18	n.e.	4.03	n.e.	6.14	6.14	5.08	5.83	6.75
3.01	3.44	3.45	3.45	3.45	4.33	4.93	3.30	3.30	3.30	3.44	3.89	4.33	3.89	4.18	n.e.	4.03	n.e.	6.14	6.14	5.08	5.83	6.75
3.15	3.59	3.59	3.59	3.59	4.48	5.08	3.44	3.44	3.44	3.59	4.03	4.48	4.03	4.33	n.e.	4.18	n.e.	6.29	6.29	5.23	5.99	6.90
3.30	3.74	3.74	3.74	3.74	4.63	5.23	3.59	3.59	3.59	3.74	4.18	4.63	4.18	4.48	n.e.	4.33	n.e.	6.44	6.44	5.38	6.14	7.05
-	0.71	0.98	0.71	0.71	1.42	2.28	0.85	0.85	1.13	1.28	1.13	1.57	1.13	1.42	n.e.	1.28	n.e.	3.74	3.74	3.45	3.45	5.23
6.42	-	1.13	0.85	0.85	1.85	2.43	0.71	0.71	0.99	1.13	1.13	1.28	1.13	1.42	n.e.	1.27	n.e.	3.74	3.74	3.01	3.01	5.38
7.14	8.54	-	0.28	0.28	1.55	2.42	0.98	0.98	0.98	1.12	0.84	1.41	0.84	0.99	n.e.	0.85	n.e.	4.17	4.17	3.74	3.86	5.66
7.26	8.25	2.71	-	0.00	1.27	2.43	0.71	0.71	0.99	1.13	0.85	1.42	0.85	0.99	n.e.	0.85	n.e.	3.74	3.74	3.74	3.30	5.23
n.e.	n.e.	n.e.	n.e.	-	1.27	2.43	0.71	0.71	0.99	1.13	0.85	1.42	0.85	0.99	n.e.	0.85	n.e.	3.74	3.74	3.74	3.30	5.23
9.26	9.98	9.68	9.69	n.e.	-	1.99	1.42	1.42	1.70	1.85	2.14	2.72	2.14	2.28	n.e.	2.14	n.e.	4.63	4.63	4.78	4.34	6.29
11.19	11.32	11.28	12.07	n.e.	6.46	-	2.57	2.57	2.57	2.72	2.72	3.02	2.72	2.87	n.e.	2.72	n.e.	5.09	5.09	3.59	4.49	6.60
9.40	9.26	11.43	11.32	n.e.	10.73	12.69	-	0.00	0.28	0.42	1.42	1.56	1.42	1.71	n.e.	1.56	n.e.	3.74	3.74	3.59	3.30	5.38
9.41	9.85	10.85	10.28	n.e.	9.26	12.55	3.29	-	0.28	0.42	1.42	1.56	1.42	1.71	n.e.	1.56	n.e.	3.74	3.74	3.59	3.30	5.38
9.12	9.26	10.56	10.28	n.e.	10.43	13.15	7.72	7.31	-	0.14	1.71	1.85	1.71	1.99	n.e.	1.85	n.e.	4.03	4.03	3.59	3.59	5.38
9.26	9.69	10.70	10.43	n.e.	10.87	13.30	8.44	8.02	0.64	-	1.85	2.00	1.85	2.14	n.e.	1.99	n.e.	4.18	4.18	3.74	3.74	5.53
10.28	11.46	11.72	11.91	n.e.	13.12	15.46	11.31	11.92	11.92	-	0.71	0.00	0.42	n.e.	0.28	n.e.	4.04	4.04	3.59	3.30	5.38	
8.82	11.31	11.27	11.31	n.e.	9.84	13.13	11.46	10.57	11.02	11.31	8.83	-	0.71	0.42	n.e.	0.85	n.e.	4.48	4.48	3.74	3.74	5.84
10.13	10.87	10.69	11.31	n.e.	12.36	14.35	10.57	11.32	11.16	11.31	5.46	8.54	-	0.42	n.e.	0.28	n.e.	4.04	4.04	3.59	3.30	5.38
8.68	10.87	9.97	9.98	n.e.	10.13	12.98	11.76	11.47	10.42	10.57	8.83	4.38	7.68	-	n.e.	0.42	n.e.	4.48	4.48	3.89	3.75	5.68
7.54	9.84	10.41	9.69	n.e.	10.42	12.68	10.87	10.57	10.72	11.02	9.27	4.77	8.54	4.91	-	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.
8.54	10.57	9.97	10.28	n.e.	10.42	13.60	11.91	11.76	9.84	10.13	8.55	6.16	8.54	5.88	6.30	-	n.e.	4.33	4.33	3.74	3.60	5.53
8.11	9.98	10.26	10.58	n.e.	10.42	13.30	11.61	11.32	10.14	10.43	7.69	5.31	8.11	5.74	6.16	2.75	-	n.e.	n.e.	n.e.	n.e.	n.e.
9.26	10.28	10.98	10.72	n.e.	11.31	13.45	11.01	11.76	9.84	9.99	12.21	11.31	11.91	10.42	10.87	9.55	9.98	-	0.00	4.04	3.15	4.93
10.28	10.72	11.86	11.76	n.e.	12.51	13.45	12.51	12.82	11.01	11.16	12.51	11.91	12.82	11.16	11.46	10.57	10.28	2.74	-	4.04	3.15	4.93
11.92	11.91	12.16	12.82	n.e.	13.89	15.45	12.66	12.97	13.12	13.58	12.66	13.43	12.37	12.66	12.66	11.76	12.51	11.76	12.06	-	2.42	4.33
9.98	10.43	12.01	11.46	n.e.	10.87	13.14	11.77	12.52	12.67	12.67	13.43	11.31	12.83	10.87	11.01	11.16	11.31	10.28	10.72	12.22	-	4.33
13.12	13.89	15.83	15.62	n.e.	14.36	16.43	14.67	14.83	14.66	14.98	15.30	14.22	15.93	14.25	14.23	13.74	13.90	12.07	11.92	15.14	14.05	-

ND5. n.e.=not examined.

*stratiotes* Borneo, 6. *G. thule* Indonesia, 7. *G. xenocles* Vietnam, 8. *G. androcles* Sulaweshi, 9. *G. nan*, 13. *G. hoenei* Sichuan, 14. *G. tamerlanum* Gansu, 15. *G. eurous* Taiwan, 16. *G. akikoe* Cameron, 21. *G. leechi* Zhejiang, 22. *G. euryptylus* Myanmar, 23. *G. agamemnon* Bali, 24. *G. wal-*  
*G. codrus* Solomons, 29. *G. empedovanum* Java1, 30. *G. sarpedon* Bali, 31. *G. milon* Sulawesi, 32.  
Camerun, 36. *G. angolanum* Bungui RCA, 37. *G. tynderaeus* Bungui RCA, 38. *G. leonidas* Bun-  
on1, 42. *L. curius* Myanmar, 43. *E. helios* Brazil, 44. *I. podalirius* Czech, 45. *T. imperialis* Vietnam

genera were not clear. The 28S NJ tree (Fig. 2a) shows that *Lamproptera* is most closely related to the genus *Graphium*, however, the 28S MP tree (Fig. 2b), the ND5 tree (Fig. 3) and the 28S+ND5 tree (Fig. 4) indicate that *Eurytides* and *Iphiclides* are more closely related to the genus *Graphium* than *Lamproptera*. Moreover, the bootstrap values of these divergences were low.

In the 28S tree (Fig. 2), the relationships among each species in the genus *Graphium* were not clear, because they were more closely related to one another and the bootstrap values were low. However, the clusters of the subgenus *Pazala* (bootstrap values NJ/MP=94/87), the *sarpedon* group (NJ/MP=86/69), the cluster of *codrus/empedovana* (NJ/MP=74/76) and the *euryptylus* group and *G. akikoe* group (NJ/MP=94/100) were recognized as monophyly with high bootstrap values, respectively. However, *G. antiphates* was not clustered with other species of the subgenus *Pathysa*.

In the ND5 tree (Fig. 3), the relationships among the groups were not clear because of low

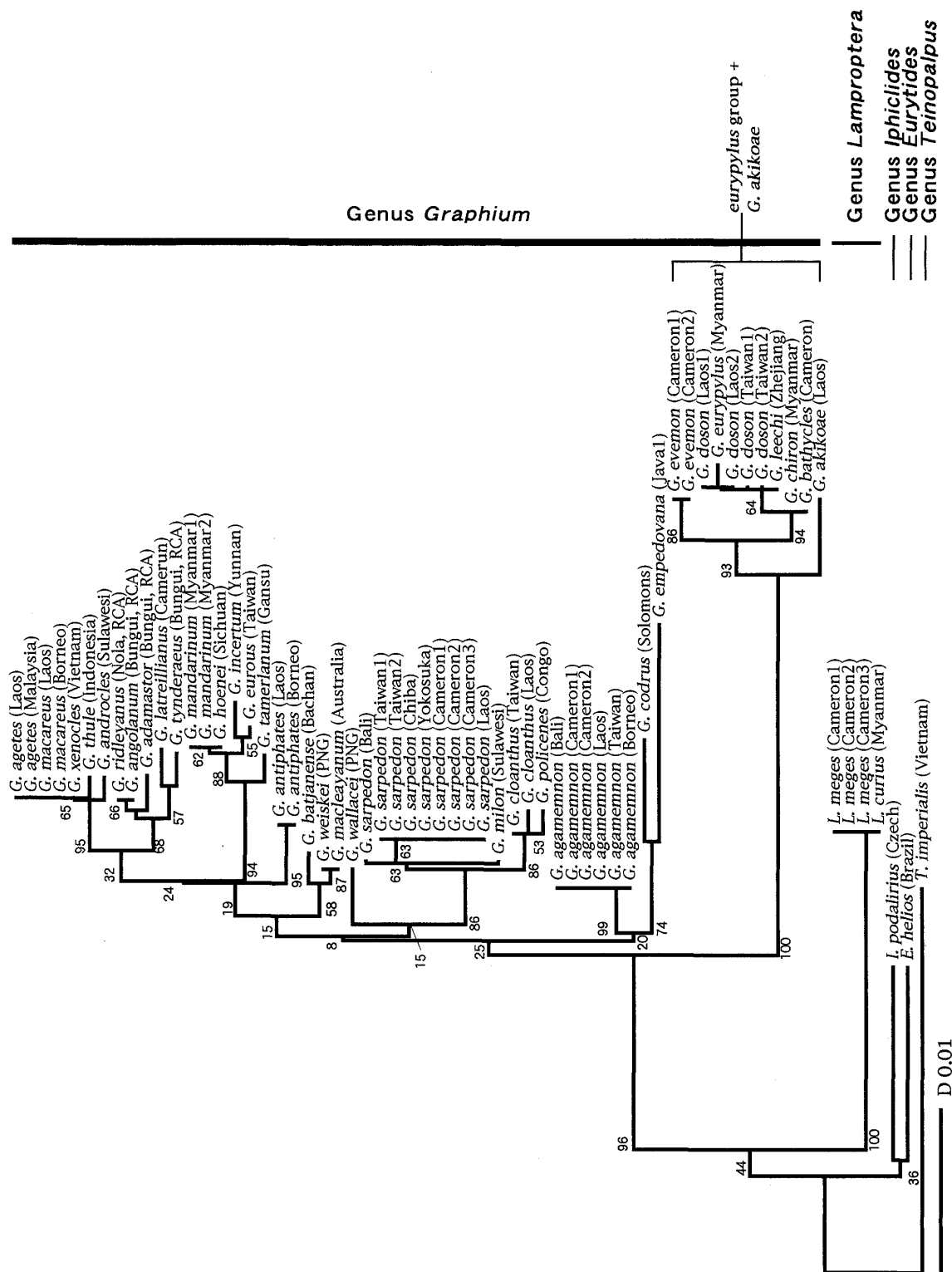
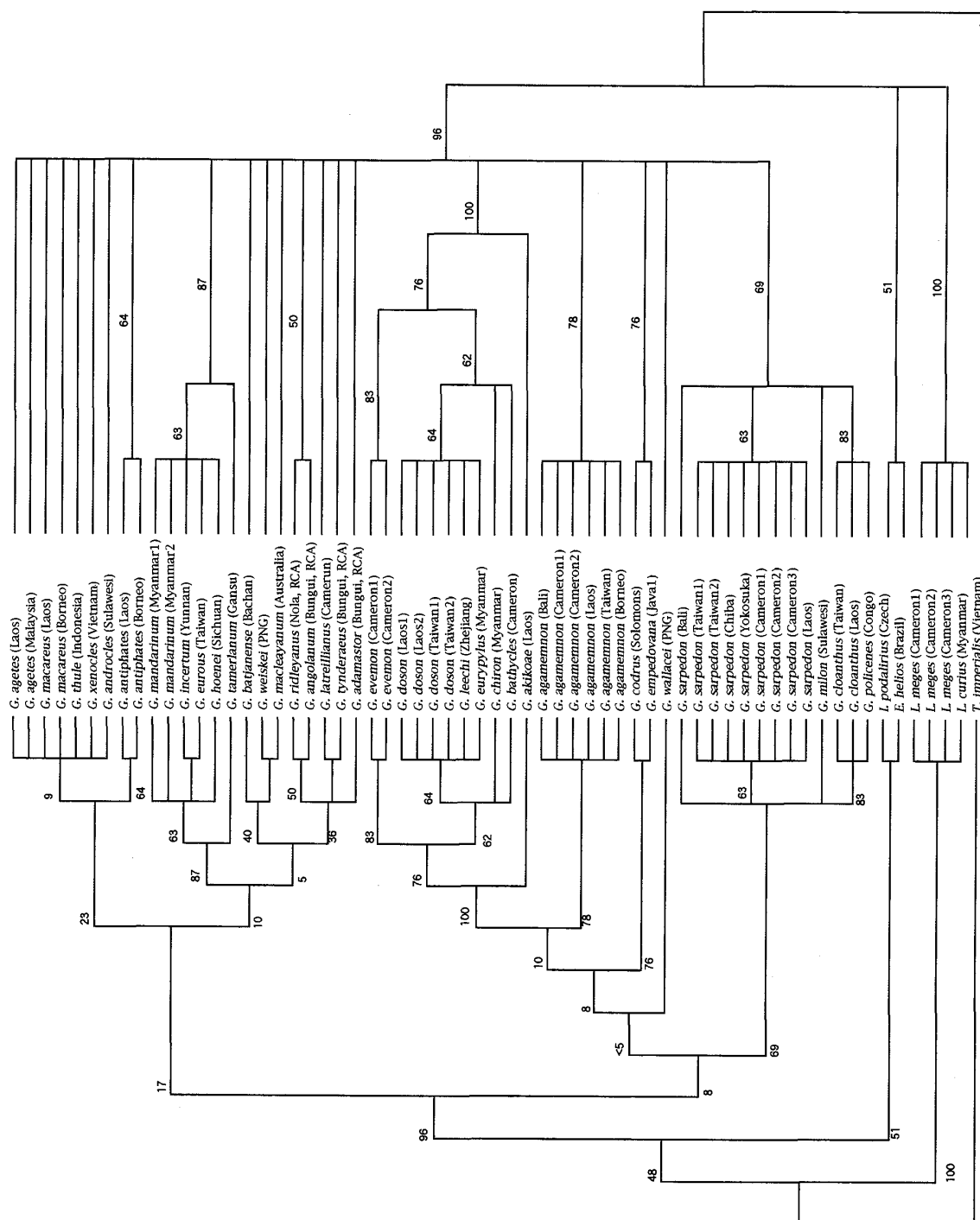


Fig. 2. Phylogenetic tree based on about 700 bases of the 28S rDNA sequences. Gaps in alignments were excluded. a: NJ tree; b: MP tree. (for Figs 2-4, a: Phylogenetic tree constructed using the neighbor-joining (NJ) method (Saitou & Nei, 1987, using PAUP\* 4.0b10, Swofford, 2000). The evolutionary distances (D) were determined by Kimura's two-parameter evolutionary distances (Kimura, 1980); b: Phylogenetic tree constructed using the maximum parsimony (MP) method (PAUP\* 4.0b10, Swofford, 2000). Heuristic parsimony analysis, all characters having equal weight, stepwise sequence



addition, addition sequence of 1,000 random replicates, tree-bisection-reconnection (TBR) branch-swapping, and the MULTREES option. The bootstrap values and majority-rule values are based on 1,000 replicates and expressed as percentages (a, b)). 2b shows one of the 217 most parsimonious trees (left) and a consensus tree of all 217 most parsimonious trees with 50% majority-rule consensus values (right). All 217 trees had a length of 162 steps; consistency index, 0.675; rescaled consistency index, 0.613; retention index, 0.909.

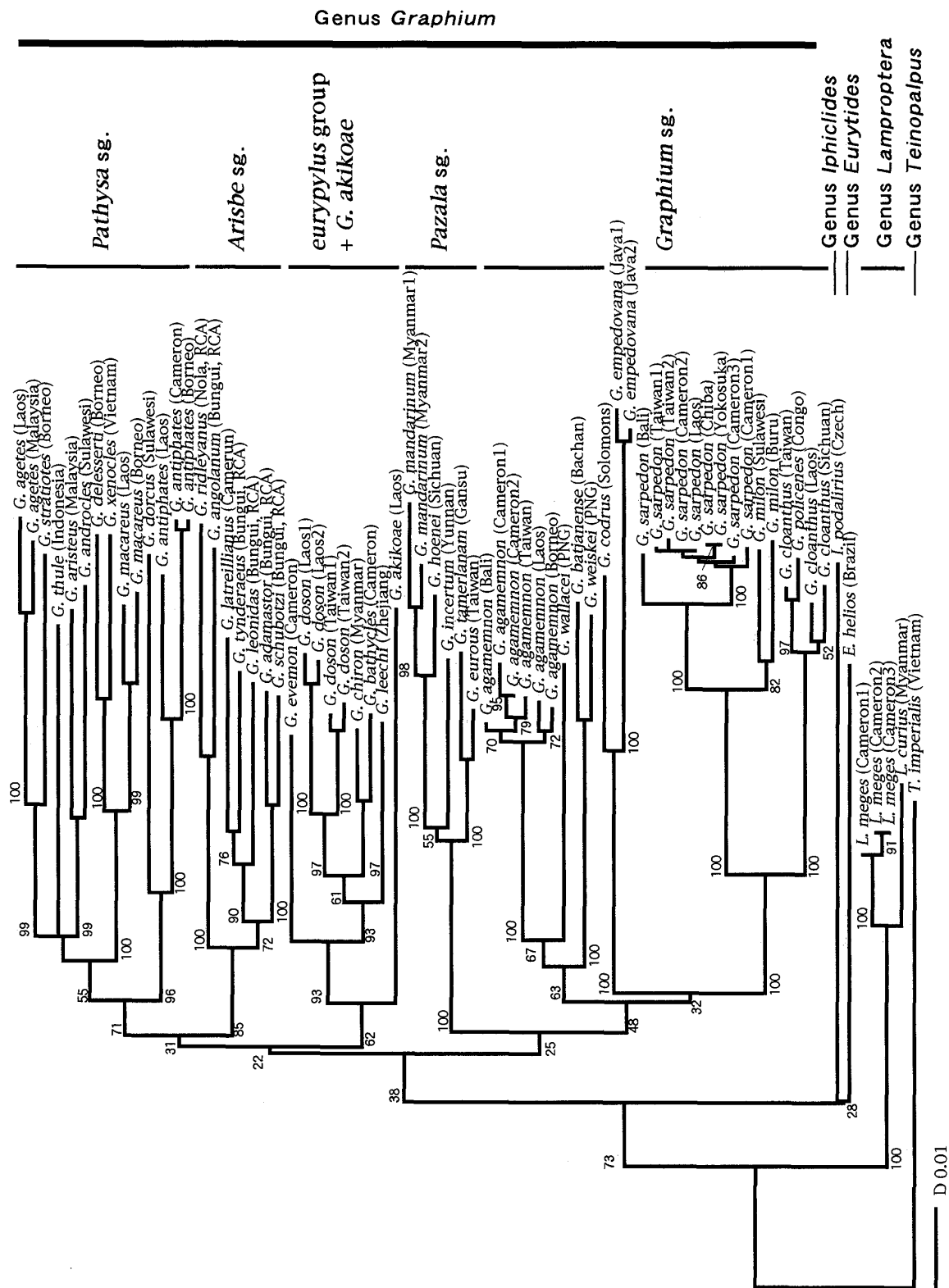
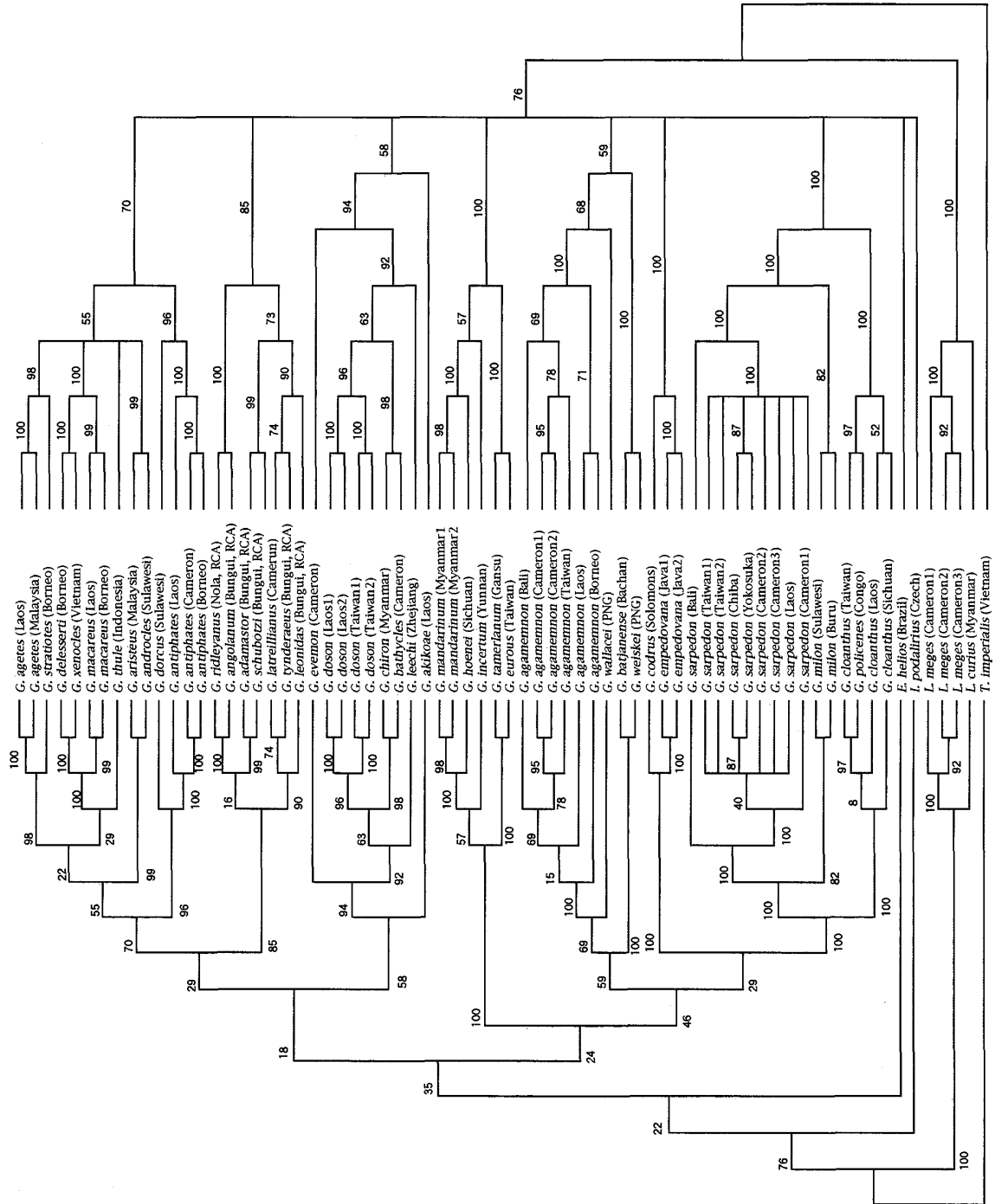


Fig. 3. Phylogenetic tree of the genus *Graphium* based on 793 bases of the mitochondrial ND5 sequences. a: NJ tree; b: MP tree. 3b shows one of the 60 most parsimonious trees (left) and a consensus tree of all 60 most parsimonious trees with 50% majority-rule consensus values (right). All 60 trees had a length of 1401 steps; consistency index, 0.344; rescaled consistency index, 0.242; retention index, 0.704.



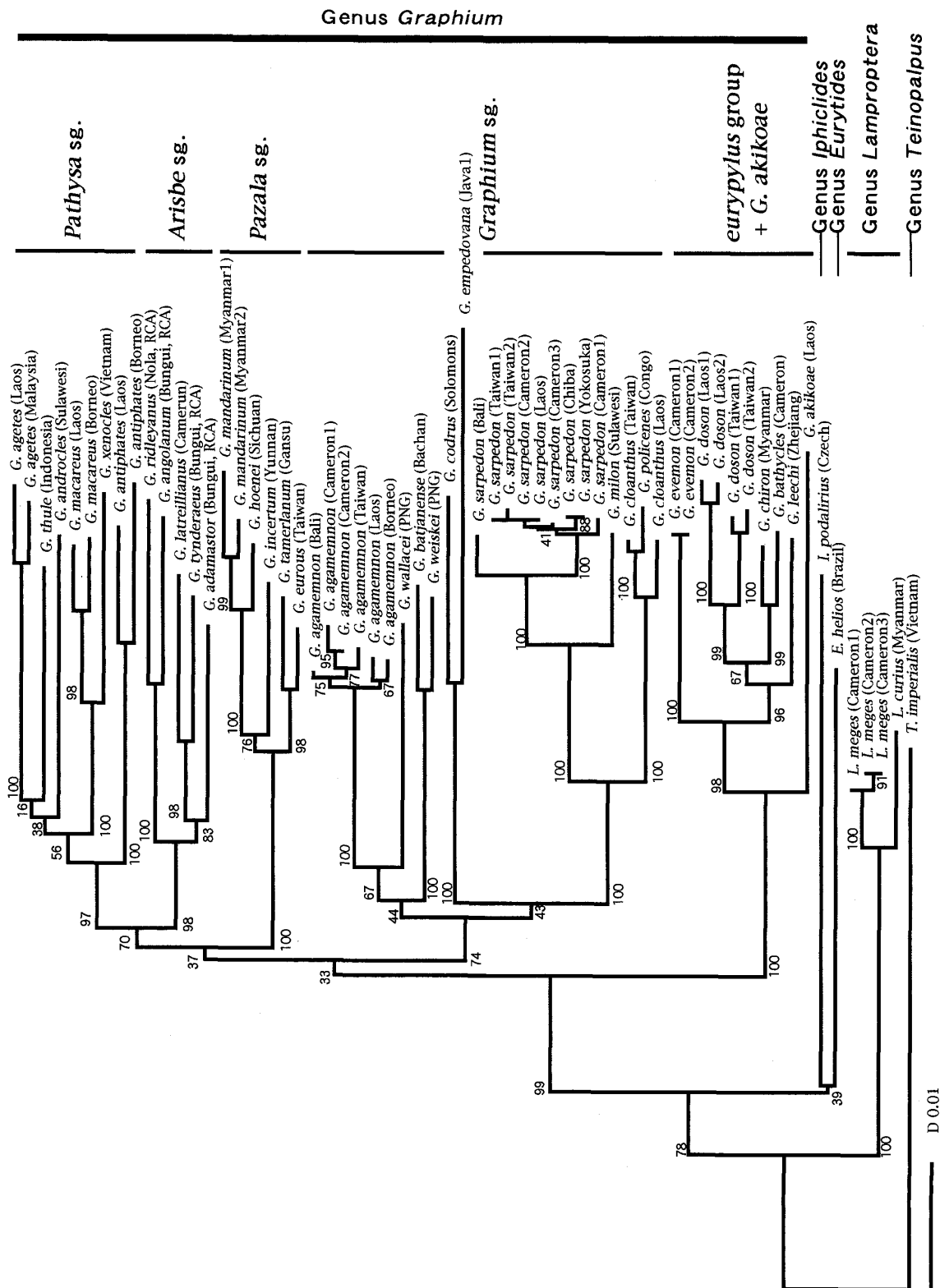
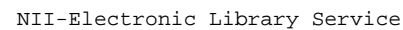


Fig. 4. Phylogenetic tree of the genus *Graphium* based on 28S rDNA and ND5 genes. Gaps in alignments were excluded. a: NJ tree; b: MP tree. 4b shows one of the 12 most parsimonious trees (left) and a consensus tree of all 12 most parsimonious trees with 50% majority-rule consensus values (right). All 12 trees had a length of 1419 steps; consistency index, 0.402; rescaled consistency index, 0.296; retention index, 0.735.



bootstrap values. The divergence of the subgenera *Pazala* (NJ/MP=100/100), *Pathysa* (NJ/MP=71/70), *Arisbe* (NJ/MP=85/85), the cluster groups of *sarpedon* and *policenes* (NJ/MP=100/100), the *agamemnon* and the *wallacei* groups and the cluster of *weiskei/batjanense* (NJ/MP=63/59), the *eurypylus* group and *akikoe* (NJ/MP=62/58) and the cluster of *codrus/empedovana* (NJ/MP=100/100) were recognized as monophyly with high bootstrap values, respectively. The *sarpedon* group, the *agamemnon* group, the cluster of *weiskei/batjanense* and the cluster of *codrus/empedovana*, which were classified into the subgenus *Graphium*, formed a monophyletic cluster. However, the *eurypylus* group was not clustered with other species of the subgenus *Graphium*.

In the 28S+ND5 tree (Fig. 4), the relationships among the groups were not clear. The divergence of the subgenera *Pazala* (NJ/MP=100/100), *Pathysa* (NJ/MP=97/63), and *Arisbe* (NJ/MP=98/82), the cluster groups of *sarpedon* and *policenes* (NJ/MP=100/98) and the *eurypylus* group and *akikoe* (NJ/MP=100/100), and the cluster of *codrus/empedovana* (NJ/MP=100/100) were recognized independently. The subgenera *Pazala*, *Pathysa* and *Arisbe* were monophyletic clusters. The cluster groups of *sarpedon* and *agamemnon*, and the cluster of *codrus/empedovana*, which were classified into the subgenus *Graphium*, formed a monophyletic cluster: however, the *eurypylus* group was not clustered with other species of the subgenus *Graphium*.

## Discussion

### Phylogenetic relationships of the tribe Leptocircini

Monophyly of the genus *Graphium* including *Pathysa*, *Pazala* and *Arisbe* is strongly supported in this study (Figs 2, 4). The bootstrap values in the 28S tree are 96 in both the NJ and MP methods, and it is also quite certain that *Pazala* and *Pathysa* are placed under the genus *Graphium*. If *Pazala* and *Pathysa* are treated as separate genera as Igarashi (1984), both *Arisbe* and the *eurypylus* group with *akikoe* should be upgraded to a generic level. However, the relationships between *Lamproptera* and the genus *Graphium* were determined to be unstable, since both the ND5 tree (Fig. 3) and the 28S+ND5 tree (Fig. 4) showed the cluster of *Eurytides* and *Iphiclides* to be more closely related to the genus *Graphium* than *Lamproptera*. The current classification suggests that *Lamproptera* is most proximate to the genus *Graphium* (Hancock, 1983; Miller, 1987). We upheld the other generic separations advocated by Munroe & Ehrlich (1960), though noting that *Iphiclides* and *Graphium* were closely related. However, the divergences between the genus *Graphium* and other genera were not definitively identified in this study, and further analysis of the genus *Graphium* is required.

### Phylogenetic relationships of the genus *Graphium*

The present classification of the genus *Graphium* includes four subgenera: *Pazala*, *Pathysa*, *Arisbe* and *Graphium* (Hancock, 1983, 1993; Miller, 1987, [Table 1]): however, our study showed no tree in which the phylogenetic relationships of the four subgenera were clear, and their diversification occurred almost at the same time (Figs 2, 3, and 4). The subgenera *Pazala*, *Pathysa* and *Arisbe* are shown as separate monophyletic clusters, but the monophyly of the subgenus *Graphium* is not supported as discussed below.

Munroe (1961) argues that the *eurous* group, placed by the later authors in the subgenus *Pazala*, belongs to the subgenus *Pathysa* based on the complexity of the male genitalia.



Hancock (1983) and Miller (1987) suggest that *Pazala* is the plesiomorphic sister group to the rest of the genus *Graphium*, and that *Pathysa* is slightly more advanced than *Pazala*. Igarashi (1984), on the other hand, raised *Pazala* and *Pathysa* to the generic level based on their immature stages. The present study shows strong monophyly of *Pazala* in the ND5 tree with high bootstrap values (NJ/MP=100/100), but its relation to the subgenus *Pathysa*, or to the subgenus *Graphium*, is not clearly shown. It is not suggested that the *eurous* group belongs to the subgenus *Pathysa*, nor is it indicated that *Pazala* is the sister group to the rest of the genus *Graphium*. In this background, it is technically probable that *Pazala* and *Pathysa* are treated as different genera if *Arisbe* is upgraded to generic level, because these subgenera were rather unstable (see Figs 2–4).

Miller (1987) proposed that all the African *Graphium* species be classified into the subgenus *Arisbe*, and that *Arisbe* formed a cluster with *Pathysa*. Hancock (1983, 1993), on the other hand, classified the African species into two groups, the non-swordtailed species in the subgenus *Arisbe* and the swordtailed species in the subgenus *Graphium*, and suggested that *Arisbe* is clustered with the subgenus *Graphium*. Smith & Vane-Wright (2001) classified the Afrotropical *Graphium* butterflies with a few Indo-Australian butterflies into the various small groups and showed complicated phylogenetic relationships, and they did not support four subgenera. The present study (see Figs 2–4) indicates that the African species are divided into two groups, one being the species belonging to *Arisbe* represented by Hancock, and the other *G. polices*. The subgenus *Arisbe* (the non-swordtailed African species) and *Pathysa* was monophyletic with high bootstrap values in Fig. 4 (NJ/MP=70/54) as proposed by Miller (1987), and *G. polices* clusters with *G. cloanthus* of the subgenus *Graphium* with high bootstrap values (NJ/MP=86/83 in Fig. 2; NJ/MP=97/97 in Fig. 3; NJ/MP=100/100 in Fig. 4) as proposed by Hancock (1983, 1993). Although we assume that the African species can be classified into the subgenera *Arisbe* and *Graphium* as in the Hancock's classification, further study of more African species may change this interpretation.

The subgenus *Pathysa* is sometimes divided into two species groups, viz. the *antiphates* group and the *macareus* group (Hancock, 1983). The former consists of some *Iphiclides*-like kite butterflies with banded forewings and long-tailed hindwings, and the latter is comprised of some mimetic butterflies with striated forewings and roundish hindwings. The present study of the ND5 gene shows that *G. delesserti*, *G. xenocles* and *G. macareus* in the *macareus* group are clustered with high bootstrap values (NJ/MP=100/100), but *G. thule* is not (Fig. 3). As mentioned below, *G. akikoe* classified into the *macareus* group by Hancock (1983) forms a cluster with the *eurypylus* group of *Graphium* outside the subgenus *Graphium*.

The *antiphates* group is shown to roughly be split into two clusters. Ostensibly similar pairs of species, such as *G. dorcus*/*G. androcles* and *G. antiphates*/*G. aristus*, are placed in different clusters with high bootstrap values in Fig. 3 (NJ/MP=97/97 in *dorcus*/*antiphates*; NJ/MP=100/100 in *androcles*/*aristus*), and the cluster of the *macareus* group excluding *thule* forms a higher cluster with one of two clusters of the *antiphates* group. The present study of 28S rDNA shows that *G. antiphates* is not clustered with other species of the subgenus *Pathysa* (Fig. 2a). However, as the 28S rDNA can not be used to determine close relationships clearly, the position of *G. antiphates* is ambiguous. The grouping of *Pathysa* by Hancock (1983) is not clearly supported in this study.

In the current classification based on Saigusa *et al.* (1977, 1982), the subgenus *Graphium* consists of the *sarpedon* group, the *agamemnon* group and the *eurypylus* group. But, Saigusa *et al.* (1977, 1982) did not specifically state that the subgenus *Graphium* is monophyletic. Miller (1987) also suggested that he could not find autapomorphic characteristics to support

the subgenus *Graphium*. The present study shows that the 28S rDNA sequence lengths of the *eurypylus* group are different from those of the other *Graphium* butterflies, and the phylogenetic tree positions of the *eurypylus* group do not cluster with these groups, but rather form a *eurypylus* cluster independently (Figs 2–4). Therefore, we assume that the *eurypylus* group does not belong in the subgenus *Graphium*. An introduction of a new subgenus will be acceptable for accommodation of this group.

Some justification of the placement of *G. akikoe* in the *eurypylus* group is required. Munroe (1961) and Hancock (1983) argued that *G. akikoe* (= *phidias*) belongs to the subgenus *Pathysa*, but *G. akikoe* is distinguished from other *Pathysa* species by various morphological structures including wing tails, forewing veins, etc. Tsukada & Nishiyama (1980), on the other hand, suggested that a new subgenus would be required for *G. akikoe*. Our results based on the sequence lengths of 28S rDNA and on our phylogenetic trees (Figs 2–4) indicate that *G. akikoe* is more closely related to the *eurypylus* group than to *Pathysa*, or to other groups of the subgenus *Graphium*. The monophyly of the cluster consisting of the *eurypylus* group and *akikoe* is supported by high bootstrap value of more than 50% in the 28S rDNA tree (NJ/MP=100/100), in the ND5 tree (NJ/MP=62/58) and in the 28S rDNA+ND5 tree (NJ/MP=100/100).

In conclusion, the present phylogenetic investigation based on molecular analysis confirmed the monophyly of the genus *Graphium*: however the phylogenetic relationships of each of the genera of the tribe Leptocircini were not made clear. These results generally agree with the traditional classification based on morphological characteristics, except for the *eurypylus* group in the subgenus *Graphium* and *G. akikoe* in the subgenus *Pathysa*. Further study of more Leptocircini butterflies, especially by adding *Protographium* and the subgenus *Eurytides* to the present material, may elucidate the relationships of each genus of the tribe Leptocircini and the subgenus *Graphium*.

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## 摘 要

核 28S rDNA とミトコンドリア ND5 遺伝子に基づく *Graphium* 属の系統解析 (牧田裕道・新川勉・近藤喜代太郎・Lianxi Xing・中澤透)

我々は、Leptocircini 族 (Graphiini 族と呼ばれることもあるが、この名称の使用については Smith & Vane-Wright (2001) 参照) または *Graphium* 属の既存の形態分類と、遺伝子配列から得られた分子系統樹の比較検討を行った。分子系統解析では、上位の系統関係は核などの進化速度の遅い遺伝子を用い、下位の系統関係はミトコンドリアなどの進化速度の速い遺伝子を用いることが報告されており、本研究では、Leptocircini 族の解析には進化速度の遅い核 28S リボソーム DNA (28S rDNA) 約 700 塩基を用いた。また、*Graphium* 属内の解析には進化速度が速いミトコンドリア NADH デヒドロゲナーゼサブユニット 5 (ND5) 遺伝子 793 塩基を用いた。分子系統樹は、NJ 法 (近隣結合法) もしくは MP 法 (最節約法) によって、28S rDNA (Fig. 2), ND5 (Fig. 3) ならびに 28S rDNA+ND5 (Fig. 4) の分子系統樹を作成し、*Graphium* 属が単系統であることが示された。また属内の各種の分岐は一斉に行われたことが示唆された。また、各亜属の系統関係は、形態分類を支持するものであったが、一部異なる結果も得られた。

形態分類では *Graphium* 属には *Pazala*, *Pathysa*, *Arisbe*, *Graphium* の 4 亜属に分類されている (Smith & Vane-Wright (2002) は 4 亜属を支持せず、複数の小グループからなるとしている)。本研究で得られた ND5 や 28S rDNA+ND5 の系統樹では、*Pazala*, *Pathysa*, *Arisbe* はそれぞれ単独のクラスターを形成した。しかし *Graphium* 亜属における *eurypylus* グループ (本研究では以下の種を解析した; *G. doson*, *G. bathycles*, *G. chiron*, *G. evemon*, *G. leechi*, *G. eurypylus*) と Hancock (1983) が *Pathysa* 亜属に分類した *G. akikoe* Morita & Shinkai (= *phidias* Oberthür) は、本研究で使用した 28S rDNA 領域の塩基数が他の亜属やグループと比較しても少なく (*eurypylus* グループと *G. akikoe* は 713 塩基, *Graphium* 属の他の種は 717 塩基), *Graphium* 亜属の他の種とはクラスターを形成しないことから、別のグループであることが分子系統樹により示され、*eurypylus* グループは *Graphium* 亜属には含まれないことが示唆された。

また、形態分類ではアフリカの *Graphium* 属を全て *Arisbe* 亜属とする説 (Munroe, 1961; Miller, 1987) と、尾状突起の無いグループを *Arisbe* 亜属 (本研究では以下の種を解析した; *G. ridleyanus*, *G. latreilianus*, *G. angolatum*, *G. tynderaeus*, *G. leonidas*, *G. adamastor*, *G. schubotzi*), 尾状突起のあるグループを *Graphium* 亜属 (*G. polices*) とする説 (Hancock, 1983, 1993) に分かれるが、本研究の分子系統樹では Hancock の分類である *Arisbe* 亜属と *Graphium* 亜属の 2 亜属に分かれることを支持している。

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